

Appl. No. 09/423,100
Supplementary Amdt. dated November 12, 2003

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Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

Claims 1-77 (canceled).

Claim 78 (currently amended): A method for preparing a protein having a correctly folded human insulin precursor, said method comprising:

expressing a recombinant protein comprising, from N-terminus to C-terminus,

a first peptidyl fragment of ~~from about~~ 20 amino acids in length to 92 amino acids in length and having an amino acid sequence which is at least 60% identical to an N-terminal amino acid the sequence of SEQ ID NO: 1 2 at least through residues 1 20 of SEQ ID NO: 1 and wherein the first fragment is capable of being bound by an anti-hGH antibody of the same length as the first peptidyl fragment or having an amino acid sequence which differs by one or two residues from the N-terminal sequence of SEQ ID NO: 2 of the same length;

an arginine or lysine residue or at least one cleavable peptidyl fragment;

a human insulin precursor peptidyl fragment comprising the human insulin A chain and the human insulin B chain;

wherein the an arginine or lysine residue or the at least one cleavable peptidyl fragment linking links the first peptidyl fragment and the human insulin precursor fragment; and

wherein the first peptidyl fragment is capable of increasing the yield of the bioactive conformation of the insulin precursor formed from contacting the recombinant protein with a chaotropic auxiliary agent as compared to the yield of the bioactive conformation of the insulin precursor formed from contacting the same recombinant protein lacking the first peptidyl fragment with the chaotropic agent; and

contacting the recombinant protein with an aqueous medium comprising the chaotropic agent,

whereby the protein is correctly folded.

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Claim 79 (previously presented): The method according to claim 78, wherein the aqueous medium comprises at least one chaotropic auxiliary agent.

Claim 80 (previously presented): The method according to claim 78, wherein the chaotropic auxiliary agent is urea.

Claim 81 (previously presented): The method according to claim 80, wherein the urea is present in a concentration between about 2 M and 8 M.

Claim 82 (previously presented): The method according to claim 81, wherein the urea is present in a concentration between about 3 M to 6 M.

Claim 83 (previously presented): The method according to claim 78, wherein the aqueous medium further comprises a mercaptan.

Claim 84 (previously presented): The method according to claim 83, wherein the mercaptan is selected from the group consisting of dithiothreitol, dithioerythrol, 2-mercaptoethanol, cysteine, methyl thioglycolate, 3-mercapto-1,2-propanediol and 3-mercaptopropionic acid.

Claim 85 (previously presented): The method according to claim 83, wherein the mercaptan is 2-mercaptoethanol.

Claim 86 (previously presented): The method according to claim 79, wherein the aqueous medium has a pH between about 8 and 10.5.

Claim 87 (previously presented): The method according to claim 79, wherein the aqueous medium has a pH between about 9 and 10.

Claim 88 (previously presented): The method according to claim 79, wherein the recombinant protein is present in the aqueous medium in a concentration between about 0.05 and 15 grams per liter.

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Claim 89 (previously presented): The method according to claim 79, wherein the recombinant protein is present in the aqueous medium in a concentration between about 0.5 and 5 grams per liter.

Claim 90 (previously presented): The method according to claim 79, wherein the recombinant protein is present in the medium in a concentration between about 2 and 3 grams per liter.

Claim 91 (previously presented): The method according to claim 78, wherein the recombinant protein is contacted with a mercaptan.

Claim 92 (previously presented): The method according to claim 91, wherein the mercaptan yields less than 5 —SH radical of the mercaptan per cysteine residue of recombinant protein.

Claim 93 (previously presented): The method according to claim 91, wherein sufficient mercaptan is provided to yield between about 0.07 to about 1.0 —SH radical of the mercaptan per cysteine residue of recombinant protein.

Claim 94 (previously presented): The method according to claim 78, further comprising isolating a portion of the expressed recombinant protein which is in the bioactive conformation.

Claim 95 (previously presented): The method according to claim 94, wherein the isolating is performed by ultrafiltration.

Claim 96 (previously presented): The method according to claim 95, wherein the ultrafiltration is performed at a pH between about 8 and 11.

Claim 97 (previously presented): The method according to claim 95, wherein the ultrafiltration is performed at a pH between about 9 and 10.

Claim 98 (canceled).

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Claim 99 (previously presented): The method according to claim 78, wherein the human insulin precursor peptidyl fragment is capable of being bound by an anti-human-insulin antibody.

Claims 100-101 (canceled).

Claim 102 (previously presented): The method according to claim 78, wherein the amino acid sequence of the human insulin precursor peptidyl fragment is the amino acid sequence of SEQ ID NO:4.

Claim 103 (previously presented): The method according to claim 78, wherein the amino acid sequence of the human insulin precursor peptidyl fragment is the amino acid sequence of SEQ ID NO:5.

Claim 104 (previously presented): The method according to claim 78, wherein the human insulin precursor peptidyl fragment consists of the A chain and B chain amino acid sequences of human insulin and therebetween a removable amino acid sequence of between 1 and 34 residues in length.

Claims 105-112 (canceled).

Claim 113 (currently amended): The method according to claim 78, wherein the amino acid sequence of the first peptidyl fragment is identical to an ~~the~~ amino acid sequence of SEQ ID NO:1.

Claim 114 (currently amended): The method according to claim 78, wherein the amino acid sequence of the first peptidyl fragment is identical to an ~~the~~ amino acid sequence of SEQ ID NO:2.

Claim 115 (canceled).

Claim 116 (previously presented): The method according to claim 78, wherein the first peptidyl fragment is between 20 and 49 residues in length.

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Claims 117-120 (canceled).

Claim 121 (previously presented): The method according to claim 78, wherein the at least one cleavable peptidyl fragment is an Arg or Lys residue.

Claim 122 (previously presented): The method according to claim 78, wherein the at least one cleavable peptidyl fragment is at least 2 amino acids in length and has a C-terminal amino acid residue selected from the group consisting of Arg and Lys.

Claim 123 (currently amended): A chimeric protein comprising from N-terminus to C-terminus:

an N-terminal first peptidyl fragment of from 20 amino acids in length to 92 amino acids in length and having an amino acid sequence which is identical to an N-terminal amino acid sequence of SEQ ID NO:2 of the same length as the first peptidyl fragment or having an amino acid sequence which differs by one or two residues from the N-terminal sequence of SEQ ID NO:2 of the same length;

a first peptidyl fragment, wherein the amino acid sequence of the first peptidyl fragment is at least 60% identical to the amino acid sequence of at least the first 20 N-terminal amino acids of SEQ ID NO:1; and wherein the first peptidyl fragment is capable of being bound by an anti-human growth hormone antibody;

an arginine or lysine residue or at least one cleavable peptidyl fragment;

a human insulin precursor peptidyl fragment consisting of a human insulin precursor which exhibits insulin-like bioactivity when folded in a bioactive conformation, and wherein the human insulin precursor peptidyl fragment comprises the A chain and the B chain of human insulin, and wherein the A chain and the B chain are separated by a removable peptidyl moiety of between 1 and 34 residues in length; and

wherein the an arginine or lysine residue or the at least one cleavable peptidyl fragment links linking the first peptidyl fragment and the human insulin precursor peptidyl fragment; and

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wherein the first peptidyl fragment is capable of mediating, upon contacting of the chimeric protein with a chaotropic agent, the formation of a correctly folded conformation of the human insulin precursor peptidyl fragment.

Claim 124 (currently amended): The chimeric protein according to claim 123, wherein the amino acid sequence of the first peptidyl fragment is an amino acid sequence of SEQ ID NO: 2 of the same length as the first peptidyl fragment.

Claims 125-126 (canceled).

Claim 127 (previously presented): The protein according to claim 124, wherein the amino acid sequence of the human insulin precursor peptidyl fragment is the amino acid sequence of SEQ ID NO:4.

Claim 128 (previously presented): The protein according to claim 124, wherein the amino acid sequence of the human insulin precursor peptidyl fragment is the amino acid sequence of SEQ ID NO:5.

Claims 129-135 (canceled).

Claim 136 (previously presented): The method of claim 78, wherein the human insulin A chain is identical in amino acid sequence to the amino acid sequence of residues 32-52 of SEQ ID NO:5 and the human B chain is identical in amino acid sequence to the amino acid sequence of residues 1-30 of SEQ ID NO:5.

Claims 137-139 (canceled).

Claim 140 (previously presented): The chimeric protein of claim 123, wherein the chimeric protein is identical in amino acid sequence to the amino acid sequence of SEQ ID NO:6 or of SEQ ID NO:7.

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Claim 141 (canceled).

Claim 142 (currently amended): The chimeric protein of claim 124, wherein the protein consists of the first peptidyl fragment; the insulin precursor ~~second~~ peptidyl fragment; and an arginine or lysine residue or the at least one cleavable peptidyl fragment; and ~~wherein the first peptidyl fragment is identical in amino acid sequence to an amino acid sequence of SEQ ID NO:1 of at least 20 amino acids in length~~ and wherein the at least one cleavable peptidyl fragment is at least 2 amino acids in length and has a C-terminal amino acid residue selected from the group consisting of Arg and Lys.

Claim 143 (previously presented): The chimeric protein of claim 124, wherein the first peptidyl fragment and the insulin precursor peptidyl fragment are linked by only one amino acid residue which is an arginine or lysine residue.